International Journal of Food Science and Technology (IJFST)
Vol. 9, Issue 2, Dec 2019, 1–12

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ASSESSMENT OF MICROBIAL QUALITY OF BALANGU-DIPPING WATER FROM SIX OUTLETS IN WUDIL TOWN IN KANO STATE, NIGERIA

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ABSTRACT

Balangu is a traditionally processed ready-to-eat meat product that is widely consumed, particularly in the Northern parts of Nigeria. One of the steps in its production involves momentarily dipping or submersion of the half-processed material in water. The aim of this study is to assess the microbial quality (bacterial and fungal loads) of the dipping water as well as to determine the characteristics of the microorganisms in order to ascertain the safety of the final balangu meat product. The microbial analysis included the total bacterial count (TBC), Coliform count, yeast and mould counts in the dipping water collected from six (6) outlets namely Tsohon Tasha, Kwanar Asibiti, KUST mini market, Sabon Gari, Bakin Kasuwa and Unguwar Fulani from Wudil Town in Kano State, Nigeria, using Standard Microbial Assessment Methods. Results obtained indicate that the mean TBC expressed as log_{10}cfu/ml ranged from 3.9 x 10^3 to 7.4 x 10^3 in the dipping water from Tsohon Tasha and Kwanar Asibiti, respectively. The thermophilic counts were found to range from 1.13 x 10^3 to 9.8 x 10^3 in the dipping water from KUST mini market and Sabon Gari outlets, respectively. The mean rope spore formers count per ml were found to range between 45 and 63 obtained from KUST mini market and Kwanar Asibiti outlets, respectively. The mean Coliform counts were found to be greater than 3 Most Probable Number (MPN)/ml in the samples obtained from Sabon Gari outlet and less than 3 MPN/ml in the other outlets. The fungal counts were found to range between 1.8 x 10^3 log_{10}cfu/mL in the dipping water from Bakin Kasuwa to 2.8x10^3 log_{10}cfu/ml in that of Kwanar Asibiti. Microscopic examinations as well as biochemical tests carried out on the different microbial isolates showed the prevalence of Staphylococcus aureus, Klebsiella spp., E. coli, Pseudomonas spp., Aspergillus niger, Aspergillus flavus, Rhizupus and Mucor in the dipping water from all the outlets. It was concluded that the high prevalence of many disease-causing organisms in Balangu-dipping water from the majority of the outlets investigated is a serious case for concern.

KEYWORDS: Balangu, Dipping-Water, Microbial, Coliform, Aspergillus & Mucor

Received: Sep 12, 2019; **Accepted:** Oct 02, 2019; **Published:** Nov 25, 2018; **Paper Id.:** IJFSTDEC20191

Original Article

INTRODUCTION

The fruit or vegetable is harvested, milk is drawn, eggs are gathered, fish and other products are obtained from natural waters and animals are collected and slaughtered, all carrying contaminating microorganisms from natural sources. In most instances, with the involvement of human handling, further contamination begins, and it continues while the product is being handled and processed.

Processed meat is any meat, which has been modified in order to either improve its taste or extend its shelf life. Methods of meat processing include salting, smoking, curing and fermentation (Ranken, 2002;

Thippareddi and Sanchez, 2006; Igwegeb *et al.*, 2019a). Processed meat products include Bacon, *Kebab*, *Suya*, *Balangu*, *Tsire*, Sausages, Corn-beef, *Danbun nama* among others. Meat processing includes all the processes that changes fresh meat with the exception of simple mechanical processes such as cutting, grinding or mixing (Igene and Ngbenebor, 1989; Heinz and Hautzinger, 2007). Processed meat products have been identified as one of the high-risk food items, with the pathogens of concern including *E. coli, Salmonella and L. monocytogenes.* There have been a large number of recalls of processed meat due to *L. monocytogenes* and significant number of food-borne illness outbreaks in which these pathogenic organisms are implicated (MacEvoy *et al.*, 2004; Min and Byoung-Kwan, 2015). There are many food-borne pathogens in the environment, which may contaminate food at any point during preparation or processing. The consumption of contaminated food could lead to fatal consequences. Biological hazards that cause food-borne illness include microorganisms, such as bacteria, viruses, fungal toxins and parasites. Bacteria are able to cause food-borne disease by infection or intoxication. Ingestion of pathogenic bacteria will result in food infection while the consumption of food containing toxins produced by bacteria such as *Staphylococcus aureus* will lead to food-borne intoxication (Muchenje *et al.,*. 2009; Olaoye, 2011). Microorganisms can contaminate processed meats during their preparation through spices, water and other ingredients as well as from processing environment, equipment and handlers that can have a significant impact on the microbiological status of the end products (MacFaddin, 2000; Vaclavik and Elizabeth, 2008). *Balangu* is one of the traditionally prepared meat products produced and widely consumed in Northern Nigeria. It is produced by spreading raw meat on butcher's paper on wire gauze, roasting or barbequing, dipping or submerging in water, and then adding seasonings and oil. *Balangu* has become very popular as a street delicacy in several countries, particularly those in West Africa. It is prepared and sold along streets often under unhygienic conditions. Most of the *Balangu* preparations and packaging materials, handlers and the surrounding environment can serve as potential sources of contamination of the products. It has been observed that most ready-to-eat meat products and the fresh meat are often prepared and displayed in Nigerian markets under poor hygienic conditions and thus are prone to contamination by microorganisms (Igwegbe *et al.*, 2019b).

One of the most important steps in *Balangu* preparation involves dipping or submerging of the incompletely processed product in water. This process involves dipping the raw meat in the water in a butcher's paper on a wire gauge with some spices or seasonings before placing the product over a smokeless fire. The processors use the dipping water for several days without proper storage and preservation before changing it; this act may lead to possible build up of particularly pathogenic microorganisms in the dipping water and therefore cause food-borne illness if the *Balangu* or the finished product is consumed. Microorganisms use water as a source and medium for their growth; therefore, water for food processing operation that comes in contact with the product in the food processing plant must meet at least the quality standard required for drinking or portable water (Potter and Hotchkiss, 1998; Alley, 2000). In many instances, quality of water higher than that suitable for drinking purposes is demanded, requiring additional treatment to ensure that all micro-organisms present are destroyed to eliminate all substances in the water that may adversely affect the appearance, taste and stability of the finished products and eventually the health of the consumers (Potter and Hotchkiss, 1998). The objectives of this study are to assess the bacteriological and fungal load of *Balangu*-dipping water from six (6) outlets from Wudil Town in Kano State, Nigeria

MATERIALS AND METHODS

Sample Collection

Wudil Town is in Wudil Local Government Area in Kano State, which is well known for raw meat, including that of cattle, sheep, goat, camel and poultry and meat product sellers. Two to three litres of the dipping water (locally known as *Ruwantsome*) were collected directly from six (6) randomly selected *balangu* processing points in Wudil Town, namely: *Bakin kasuwa* (A), KUST mini market (B), *Kwanari Asibiti* (C), *Sabon Gari* (D), *Tsohon tasha* (E) and *Unguwar* Fulani (F) using previously sterilized plastic containers. The sampling method used is as described by Horwitz (2010) and Gomes *et al.* (2010). All samples were aseptically transported to the microbiology and food analysis laboratories in the Department of Food Science and Technology, Kano University of Science and Technology (KUST), Wudil, for the analyses.

Sample Preparation

Samples were handled and prepared according to the methods described by Levine *et al.* (2001). Twenty five (25) milliliters of the dipping water from each of the outlets were transferred into 225 ml of sterile peptone water. The samples were shaken thoroughly to obtain homogeneous mixtures (these served as stock solutions for each outlet). Serial dilutions were made using 1 ml from the stock homogenate and 9 ml of sterile distilled water. Several dilutions were made, up to 5 folds (10^{-5}) for each outlet, in order to obtain discrete colonies.

Preparation of Media

The following media: potato dextrose agar (PDA) for mold count, nutrient agar (NA) for total aerobic plate count, Mannitol salt agar (MSA) for staphylococcus, eosine methylene blue agar (EMBA) for yeast and desoxycholate citrate agar (DCA) for Salmonella/Shigella were used in this study; they were used for the enumeration of bacteria, yeast and mold as well as for pure culture selection of the microorganisms. All glassware, including petri-dishes, test tubes, pipettes, flasks and bottles used in the analysis were sterilized in a hot oven at 170 ± 5°C for at least two hours, while the media and distilled water were sterilized by autoclaving at 121°C for 15 min and at 15 psi (Igwegbe *et al*., 2014). Each medium was prepared following the manufacturer‘s instruction. Plating was carried out in triplicate and pour plate method was used to make the viable counts (Quinn *et al*., 2002;Vipul *et al*., 2012; Igwegbe *et al.*, 2019b). In this method, one (1) ml of the inoculums was mixed thoroughly in molten plate count agar held in a hot water bath at 47 ± 2°C. The agar was allowed to set; the plates were inverted and then incubated at 32°C for 24 – 48 hours for bacterial counts (including mesophilic and thermophilic spore formers) and at 25°C for 5 - 7 days for yeast and mold counts, while the coliform count (MPN/ml) were determined using 3-tube MPN techniques (APHA, 1992; Townsend *et al.*, 1998; Quinn *et al*., 2002; Cheesbrough 2006). For each dilution, the viable colonies, which appeared colorless, in the three plates were counted and the means were calculated.

Isolation of the Microorganisms

The isolation of *Escherichia coli* was achieved following the methods described by American Public Health Association (1992) and Townsend *et al.* (1998); that of *Staphylococcus aureus* and *Staphylococcus epidermidis* were by the methods described by Stanley *et al.* (2015) while *Pseudomonas aeruginosa* and *Klebsiella spp.* were isolated by the methods described by MacFaddin (2000). The isolated organisms were further subjected to Gram-staining technique as described by Cheesbrough (2006).

Test for Coliform using the 3-tube (MPN) Technique

Twenty-five (25) ml of the dipping water was aseptically transferred into 225 ml of sterile lactose broth, which served as stock. Serial dilutions were made in order to arrive at up to 3-fold (10^{-3}) for each prepared sample adding 1 ml of the previous dilution (the stock) to 9 ml of the sterile lactose broth. Three (3) replicate tubes containing Durham tubes and lactose broth per dilution were prepared with 1 ml of previously prepared 10^{-1} to 10^{-3} dilutions. The tubes were then incubated for 24 hours at 35^0C and were examined for gas production. Negative tubes were incubated for additional 24 hours. All tubes showing evidence of gas production within 48 ± 2 hours were recorded, and the number counted was referred to as the most probable number (MPN) for the three tubes dilution (Cheesbrough, 2006).

Microscopic Examination and Identification Colonies

The characterization and identification of the colony isolates were achieved by initial morphological examination of the colonies in the plate for colonial appearance, size, elevation, form, edge, color and odor and the results were recorded, this was done for both bacterial and fungal isolates from their respective media. The biochemical tests including catalase, coagulase, citrate utilization, oxidase, urease, Voges-Proskauer, motility, sugar fermentation, methyl red and indole production tests were carried out as described by Cheesbrough (2006).

Characterization of Fungal Isolates

The fungi were identified using lactophenol cotton blue technique. A drop of lactophenol cotton blue was placed on the grease free slide. A straight wire loop was used to pick the organism from the colony and traced on the drop. A cover slip was placed on the lactophenol cotton blue and examined under X4 objective lens to check for the morphological characteristic of the organisms (Cheesbrough, 2006).

Statistical Analysis

The results obtained in this study were subjected to Analysis of Variance (ANOVA) to determine differences in microbial loads between the six outlets. The test for significance among means was conducted using the Duncan's Multiple Range Test at 5% levels of significance (Montgomery, 1976).

RESULTS AND DISCUSSIONS

In all food processing activities, there is the overriding concern to avoid food poisoning. Food is the only commodity that people buy every day and take into their bodies. Processors and processing methods must meet strict standards of cleanliness and production control to avoid the risk of harming or even killing their customers by allowing the presence and growth of food poisoning organisms in their products. Although small-scale food producers often start by working from home using domestic equipment; they often have little money to invest in equipment and little access to credit; however, they must be able to produce uniform quality foods under hygienic conditions. Acidic foods (such as yoghurt, pickles, fruit juices, jams) and most types of dried foods have a low risk of transmitting food poisoning microorganisms. In contrast, low-acid foods such as meat, milk, fish and some vegetable products are much more susceptible to transmitting food-borne illness through poor hygiene of workers or incorrect processing conditions. The results obtained in this study have shown the presence of significant number of microorganisms in the dipping water from all the six outlets in Wudil Town (**Table 1**). The highest total bacterial count (TBC), 7.4 x 10^3 $\log_{10}$cfu/ml, was recorded in dipping water from outlet C followed by that of outlet D, 6.4 x 10^3 $\log_{10}$ cfu/ml, and both of them are significantly different ($p \geq 0.5$) from those of

other outlets; whereas, the least TBC recorded, 3.9 x 10^3 log_{10}cfu/ml, was in dipping water from outlet E followed by 5.0 x 10^3 log_{10}cfu/ml in B (**Table 1**).

Table 1: Total Bacterial and Fungal Counts of the Balangu-Dipping Water from the Six Outlets[1]

Outlets[2]	Types of Organism[3]				
	TBC (Log_{10}cfu/ml)	TC (Log_{10}cfu/ml)	Rope Spores (Count/ml)	Coliform (MPN/ml)	TFC (Log_{10}cfu/ml)
A	6.0 x 10^3	8.90 x 10^3	57	<3	1.8 x 10^3
B	5.0 x 10^3	1.13 x 10^3	45	<3	2.4 x 10^3
C	7.4 x 10^3	9.70 x 10^3	63	<3	2.8 x 10^3
D	6.4 x 10^3	9.80 x 10^3	47	<3	1.9 x 10^3
E	3.9 x 10^3	9.00 x 10^3	55	<3	2.0 x 10^3
F	6.3 x 10^3	6.30 x 10^3	54	<3	2.4 x 10^3

[1]Values are means of triplicate readings;

[2]A = Bakin Kasuwa, B = KUST mini market, C = Kwanar Asibiti, D = Sabon Gari, E = Tsohon Tasha, F = Unguwar Fulani;

[3]TBC = total bacterial count, TC = thermophilic count, MPN = most probable number, TFC = total fungal count.

Similarly, the thermophilic count (TC) was the highest in outlet D, 9.8 x 10^3 log_{10}cfu/ml, followed by 9.7 x 10^3 and 9.0 x 10^3 log_{10}cfu/ml in outlets C and E, respectively, and the least TC, 1.13 x 10^3 log_{10}cfu/ml, was observed in outlet B. The rope spore formers were highest in outlet A, 57 counts/ml, followed by E and F, 55 and 54 counts/ml, respectively, and the least, 45 and 47, were recorded in B and D, respectively (**Table 1**). On the other hand, no significant numbers ($p \leq 0.5$) of Coliform (MPN/ml) were recorded in all the outlets investigated. Also, the fungal counts were recorded but not in significant numbers except for outlets A and D, 1.8 x 10^3 and 1.9 x 10^3 log_{10}cfu/ml, respectively, which are the least in fungal counts (**Table 1**). Also, results of morphological and biochemical characterization of the bacterial and fungal isolates from the *balangu* dipping water from the six outlets are presented in **Tables 2** and **3,** respectively. The results revealed the presence of four different bacterial species, namely, *Staphylococcus aureus*, *Psuedomonas spp., Klebsiella spp.* and *E. coli* (**Table 2**), and four fungal species including *Mucor, Rhizopus stolonifer, Aspergillus flavus and Aspergillus niger* (**Table 3**)*,* from most of the outlets. Furthermore, the prevalence of bacteria and fungi from the various *balangu*-dipping water samples were investigated and calculated based on the number of colonies obtained. **Tables 4** and **5** indicate high percentage in prevalence of *Staphylococcus aureus, Psuedomonas spp., Klebsiella spp. and E. coli* colonies in the dipping water from four of the outlets, namely, A, D, E and F (**Table 4**); whereas high percentages of *A. niger, A. flavus, Rhizopus stolonifer and Mucor* were recorded in the dipping water from C, E and F (**Table 5**). The muscles of healthy animals are generally considered as sterile, but the slaughtering and butchering process of meat animals provide microorganisms such as bacteria, mold and yeasts, with an opportunity to colonize meat surfaces. In addition, contamination of meat is a continuing possibility from the moment of bleeding until consumption. Starting from the abattoir itself, there are many potential sources of contamination of meat by microorganisms. These include the animal hide, hair and soil adhering thereto, the contents of the gastrointestinal tracts (especially in the event of accidental bursting of the stomach and tripe during the dressing operations), airborne contamination, the water used for washing the carcass or for cleaning the floors and equipment, the instruments used in dressing the carcass (such as knives, saws, cleavers and hooks), various vessels and receptacles and the handlers or personnel (Yousuf *et al.*, 2008; Muchenje *et al.*, 2009; Olaoye, 2011; Igwegbe *et al.*, 2019b). Also, aerosols generated during deskinning, evisceration and carcass splitting could be important sources of contamination (Mead, 2004; Meng *et al.*, 2007).

Table 2: The Morphological and Biochemical Characterization of Bacterial Isolates from *Balangu*-Dipping Water Investigated in this Study

Colony Morphology	Microscopic Observation	Oxidase	Catalase	Coagulase	Indole	Motility	Citrate	Methylred	Acid prod	VP	Suspected Organism
Opaque Cream yellow Growth	Gram Positive Cocci in Clusters	-	+	+	-	+	-	+	-	+	Staphylococcus aureus
Shiny mucoid /viscous Colonies	Gram Negative Short Rod	-	+	-	+	+	-	+	+	-	Klebsiella spp.
Green Metallic Sheen colonies	Gram Negative Rods	+	-	+	+	-	+	-	-	+	E. coli
Greenish Gray Colonies with convex Elevation and irregular Ends	Gram Negative Rods in singles, some in pairs	+	+	-	-	+	+	-	-	+	Pseudomonas spp.

Key: + = Positive Test; - = Negative Test; VP = Voges-Proskauer,

Table 3: Morphology and Microscopic Appearance of Fungal Isolates obtained in this Study

Isolates	Morphological Description of Colonies	Microscopic Appearance	Suspected Organism
1	White cottony at first then grey as it grows older	Sporangiophores arise singly from mycelium at any point. All branches terminate in sporangia	Mucor
2	Black dusty and spongy	Sporangiophores arise from long-arching stolons opposite rhizoids	Rhizopus stolonifer
3	Yellow green surface with reddish brown underneath	The apex of the conidiophores is swollen into a vesicle from which arises bottle-shaped cells, sterigmata which bear chains of globose conidosphores	Aspergillus flavus
4	Dark-brown to black pigmentation	Numerous mycelia conidiophores are black, spherical to oval, produced in long chains.	Aspergillus niger

Stanbridge and Davies (1998) reported that air circulated from heavily contaminated refrigeration coils in poultry processing plants was a major source of contamination of the products by microorganisms. Generally, the initial microbial load of a carcass surface is determined by the hygienic condition of the abattoir as well as the prevailing handling practices as well as processing methods (Guerrero *et al.*, 1995; Ranken, 2002, Rahman *et al.*, 2005). This study has confirmed the findings of Mor-Mur and Yuste (2010) and Olaoye (2011), which stated that many food borne diseases are associated with consumption of meat, and that some of the meat carcasses on sale as well as many ready-to-eat meat products hawked along the streets and major roads in developing countries, including Nigeria, are contaminated with one type of pathogen or another. The assessment of some pathogenic microorganisms can serve as a good indicator of the microbiological quality of the meat products. The mesophilic aerobic flora has been used as a guide to predict the mean shelf life of a product. Food borne diseases are among the major health problems that causes high number of morbidity and mortality each year (Hanson *et al.,* 2012).

Table 4: Prevalence of Bacteria (%) in Balangu-Dipping Water from the Six Outlets[1]

Outlet[2]	S. Aureus		Pseudomonas spp.		Klebsiella spp.		E. coli	
	N	%	N	%	N	%	N	%
A	21	39.6	19	35.9	13	24.5	0	0
B	17	40.4	12	28.6	13	31	0	0
C	31	43.7	22	30.9	18	25.4	0	0
D	33	45.8	25	34.7	12	16.7	2	2.8

E	28	52.8	11	20.8	14	26.4	0	0
F	12	27.9	14	32.6	17	39.5	0	0

[1]N = Number of isolates;

[2]A = Bakin Kasuwa, B = KUST mini market, C = Kwanar Asibiti, D = Sabon Gari, E = Tsohon Tasha, F = Unguwar Fulani.

These pathogens can be used as indicators of inadequate product manufacturing procedure and post-processing handling. This study has dwelt on the isolation, characterization and identification of bacterial and fungal colonies from *balangu*-dipping water from six different outlets in Wudil Town, Kano. The findings are in line with those of El-Gamal *et al.*, (2014) and Ameme *et al.* (2016) in similar studies in Egypt and Gomes *et al.* (2010) in Portugal. Also, the isolation of *Staphylococcus aureus, Klebsiella spp., E. coli, Psuedomona ssp., Mucor, Rhizopus stolonifer, Aspergillus niger* and *Aspergillus flavu*s was in agreement with the observations of Nichols *et al.* (1999), Mensah *et al.* (2002) and Oranusi and Braide (2012) that these microorganisms were implicated in food-borne diseases caused by ready–to-eat foods. The presence of fungi such as *Mucor, Rhizopus stolonifer, Aspergillus niger* and *Aspergillus flavus* in the dipping water may be as a result of the contamination by dust and soil, as they disperse in the form of spores, which are abundant in the *balangu* processing environment.

Table 5: Prevalence of Fungi (%) in Balangu-Dipping Water from the Six Outlets[1]

Outlet[2]	**A. Niger**		**A. Flavus**		**Rizorphus Stolonifer**	**Mucor**		
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
A	9	24.3	6	16.2	14	37.8	8	21.6
B	7	21.9	5	15.6	11	34.4	9	28.1
C	11	28.2	7	17.9	9	23.1	12	30.8
D	6	26.1	2	8.7	9	39.1	6	26.1
E	8	26.7	1	3.3	12	40.0	9	30.0
F	5	15.2	4	12.1	11	33.3	13	39.4

[1]N = Number of isolates;

[2]A = Bakin Kasuwa, B = KUST mini market, C = Kwanar Asibiti, D = Sabon Gari, E = Tsohon Tasha, F = Unguwar Fulani.

Presence of *Mucor, Rhizopus stolonifer, Aspergillus niger* and *Aspergillus flavus* in particularly ready-to-eat meat products is of great public health concern, as these organisms have been implicated in production of carcinogenic mycotoxins (Ameme *et al.,* 2016). Also, the presence of thermophilic and rope spore formers recorded in this study is in agreement with those reported by Milliotis and Bier (2003), who also confirmed the sources of these organisms to be dust, soil, raw foods and that they can survive normal cooking as heat resistance spores. The heat-resistant spores may survive cooking, while the vegetative parts are denatured by heat. The presence of *Klebsiella spp. and E. coli* may be as results of fecal contamination, as suggested by Adams and Moss (2008). The detection of *Staphylococcus aureus, Klebsiella spp., E. coli, Psuedomona ssp., Mucor, Rhizopus stolonifer, Aspergillus niger* and *Aspergillus flavus* demonstrates the potential health risk that may be associated with consumption of the *balangu* meat from those spots. Therefore, it is mandatory that food should be free from such contaminating organisms as much as possible as recommended by Wagner, (2009) and Osamwonyi *et al.,* (2013). Based on the International Commission for Microbiological Safety of Foods (ICMSF), the microbial counts recorded in this study were, however, within the acceptable limits. Moreover, foodborne illness can be

perfectly prevented by good hygienic practices, such as the adoption of Good Manufacturing practices (GMP) and Hazard Analysis and Critical Control Point (HACCP) in the production of *balangu* meat products.

CONCLUSIONS

The microbiological load of *balangu*-dipping water obtained from the various outlets in Wudil Town reveals the presence of *Staphylococcus aureus, Klebsiella spp., E. coli, Psuedomona ssp., Mucor, Rhizopus stolonifer, Aspergillus niger* and *Aspergillus flavus* which demonstrates the potential health risks that may be associated with eating the *balangu* meat. Though the microbial counts do not exceed the permissible range recommended by the International Commission for Microbiological Safety of Foods (ICMSF), the incidence of pathogenic organisms in ready-to-eat meat products like *balangu* should be of serious health concerns.

ACKNOWLEDGMENT

The authors highly acknowledge the Department of Food, Science and Technology, University of Maiduguri, Borno State; Department of Food Science and Technology, Federal University Dutsin-ma, Katsina State and Department of Biochemistry Federal University Dutse, Jigawa State for providing resources and conducive research facilities used in this study.

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